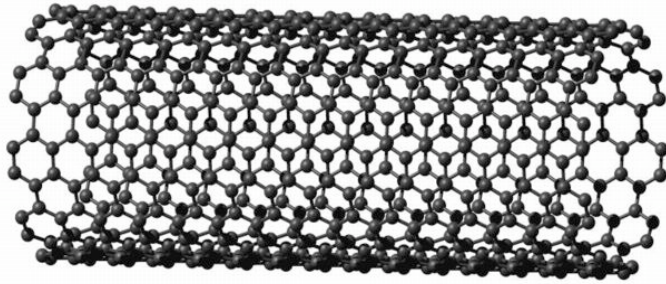


What Do We Know About The Potential Toxicity of Inhaled Carbon Nanotubes?

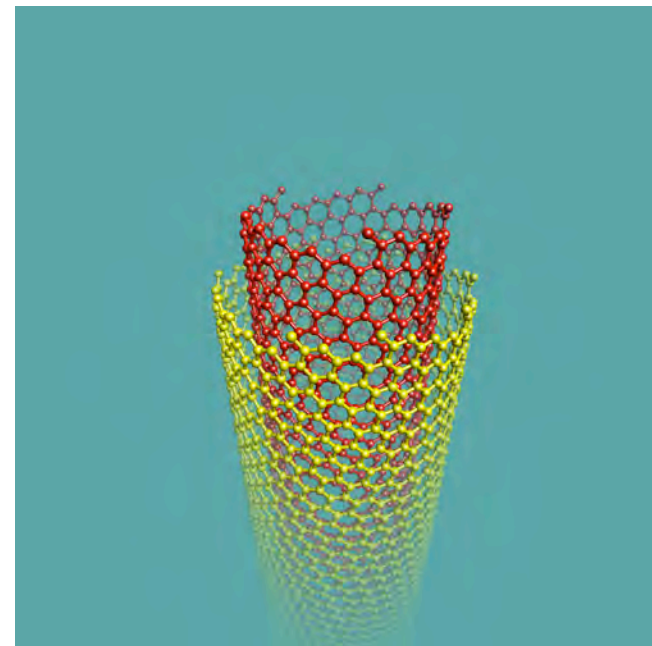
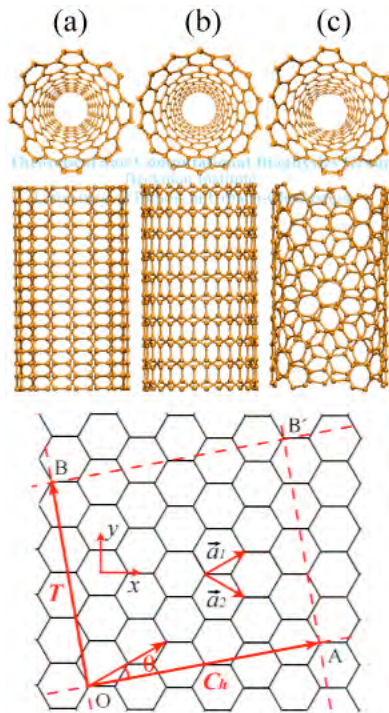
Rick Kelly

**Facility and EH&S Manager
Materials Sciences Division and
The Molecular Foundry**

Carbon Nanotubes



•Single wall carbon nanotube

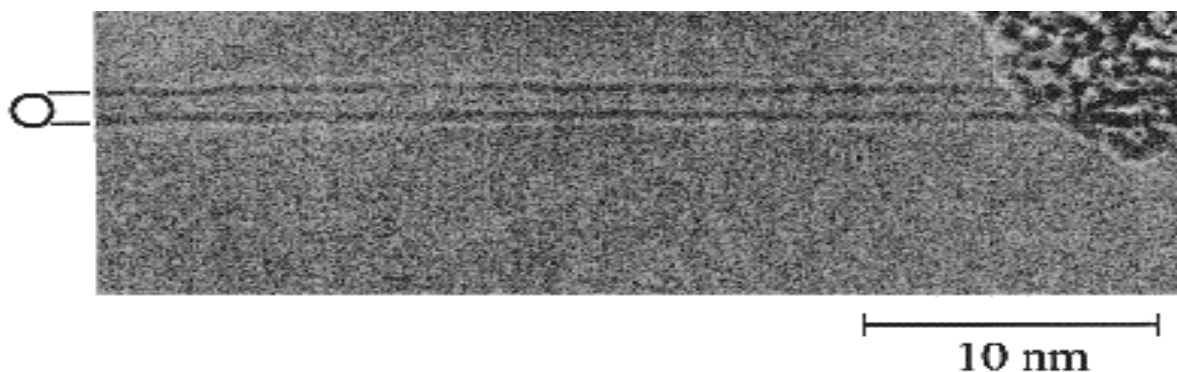


Multiwall carbon nanotube

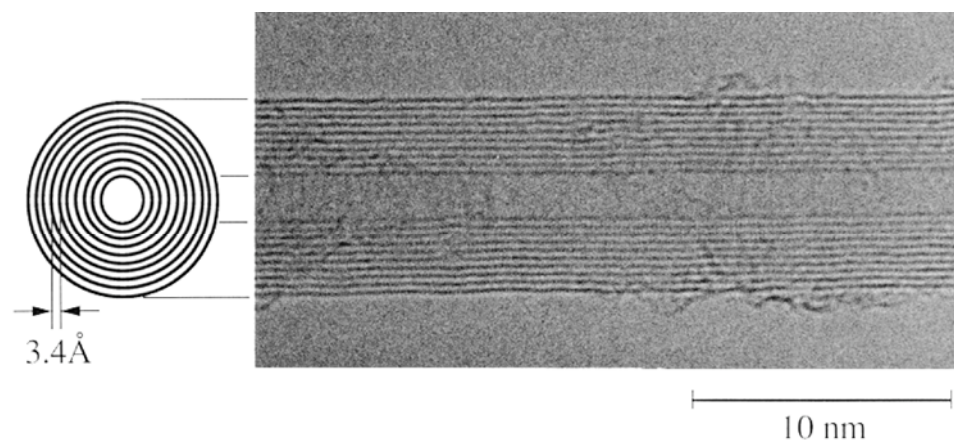
Single Tube Electron Micrographs



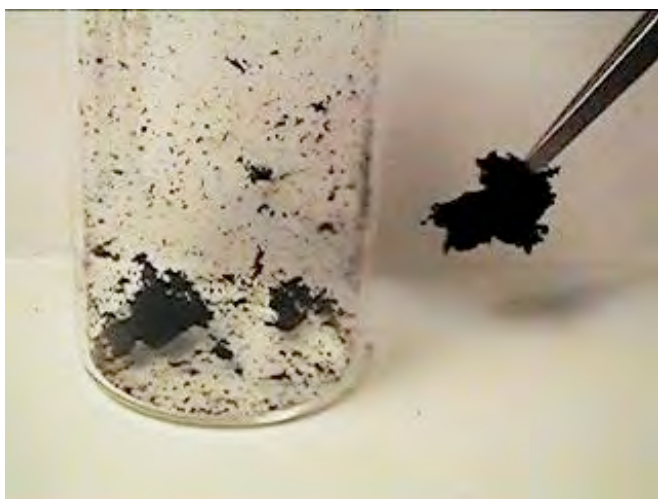
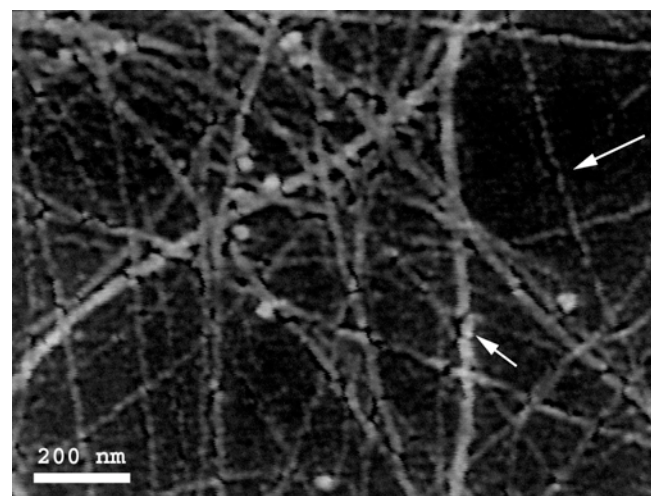
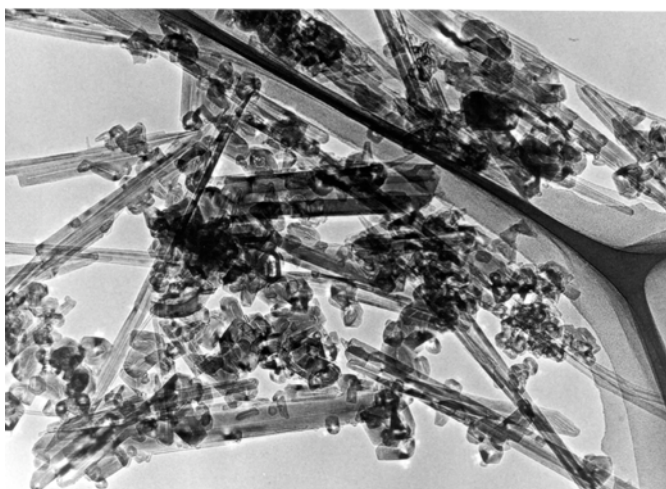
Single-Walled Carbon Nanotubes (SWCNTs)



Multi-Walled Carbon Nanotubes (MWCNTs)



What CNTs really look like!



- Clumps, ropes, bundles, mats
- Residual catalyst, other carbon forms
- Very high tendency to stick together

Why Do We Care?



- **Properties of nanoscale materials may be fundamentally different from bulk materials**
- **Among the new properties of nanoscale materials may be:**
 - **New toxicological properties**
 - **New environmental hazards**

People Are Concerned!



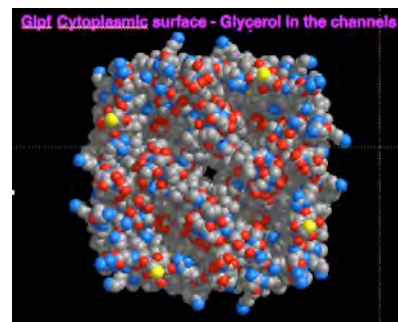
- Protesting Eddie Bauer stain resistant “Nanopants”



CNT Can Be “Free Engineered Nanoscale Particulate Matter”



- Not firmly attached to a surface
- Not part of a bigger item (e.g., wafer, cell wall)
- Can result in exposure via inhalation, skin absorption or ingestion



Handling nanotube material

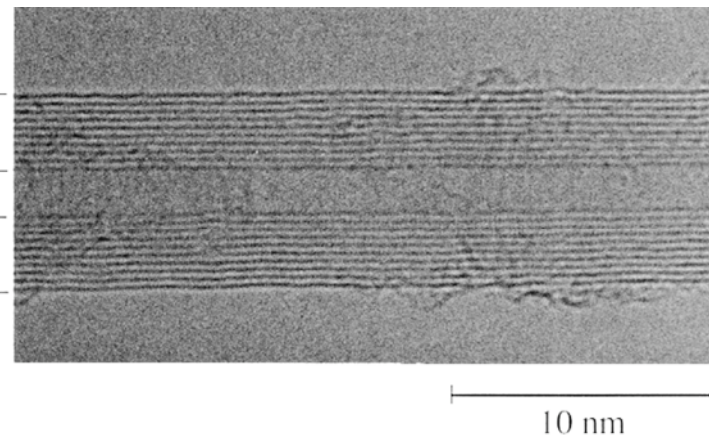
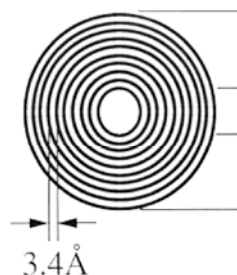
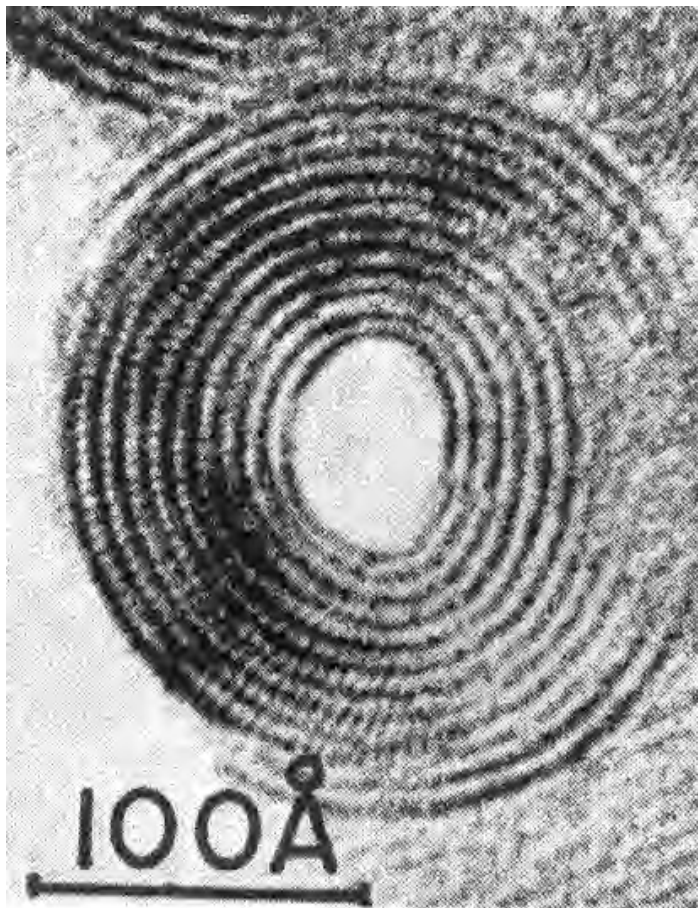


Material removal from High pressure
CO disproportionate process (HiPco)
(CO over $\text{Fe}(\text{CO})_5$)



Removing material
from laser ablation
reactor

Multiwall Carbon Nanotubes Look A Lot Like Chrysotile Asbestos



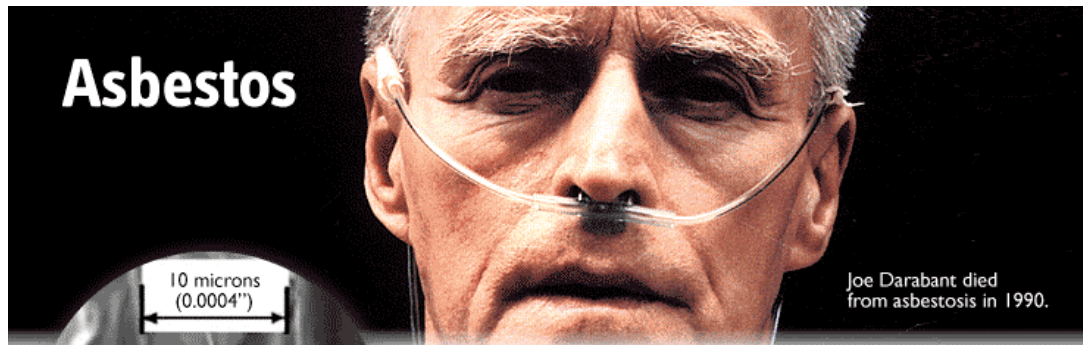
Two similar appearing nanofibers

- Chrysotile asbestos (left)
- Multiwall carbon nanotube (above)

Similar toxicity?

www.gly.uga.edu/schroeder/geol6550/CM07.html

Chrysotile Asbestos: Your Father's Nanotube?



You'd think... a substance that kills 10,000 Americans each year would be banned.

You'd think... that Congress would do everything possible to help those afflicted with asbestos diseases.

Think again.

Photograph of Joe Darabant
Copyright Bill Ravanesi | [About the photographer](#)

[click for next page]

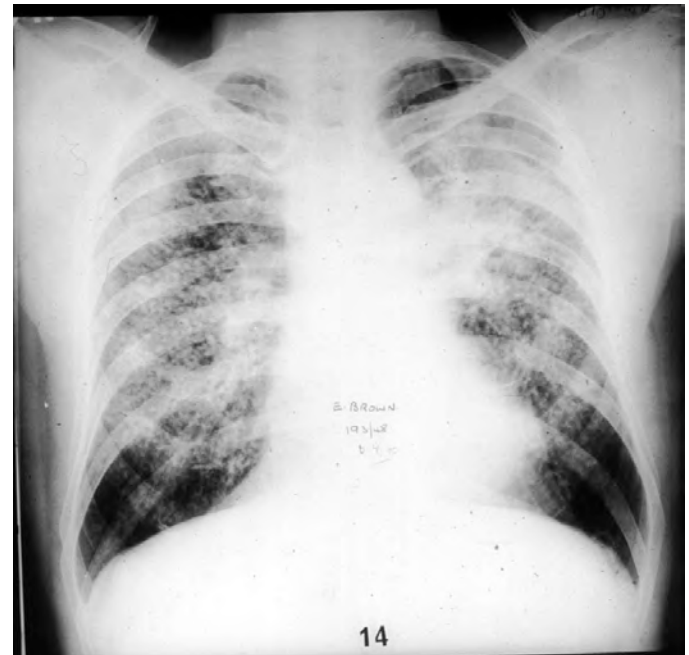


- $\text{Mg}_3\text{Si}_2\text{O}_5(\text{OH})_4$ rolled sheet fiber
- Causes lung fibrosis, lung cancer and mesothelioma, maybe other cancers

What is pulmonary fibrosis?



- **Pulmonary Fibrosis—scarring of the lung.**
- **Air sacs of the lungs become replaced by scar tissue.**
- **Irreversible loss of the ability to transfer oxygen to blood.**
- **Diseases such as Scleroderma, Rheumatoid Arthritis, Lupus and Sarcoidosis**



What is mesothelioma?



- **Mesothelioma is a cancer of the mesothelium, the protective sac that covers most of the body's internal organs.**
- **Can involve lining of lungs, heart, gut**
- **Mesothelioma is a rare disease, in the US ~80% is associated with exposure to asbestos**
- **Not caused by cigarette smoking**
- **~100% fatal, average survival is about 18 months**

Fiber Toxicology



- Many naturally occurring and man-made fibers can induce mesothelioma, lung cancer and/or pulmonary fibrosis:

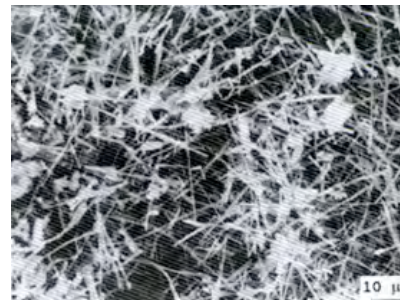
- Fibrous erionite & zeolite: High rate of mesothelioma in the Anatoly region of Turkey where they occur naturally—more potent than asbestos

- Man made vitreous fibers

- Man made refractory ceramic fibers

- Silicon carbide whiskers: Similar potency to asbestos

- Aluminum oxide, attapulgite, dawsonite, potassium titanate

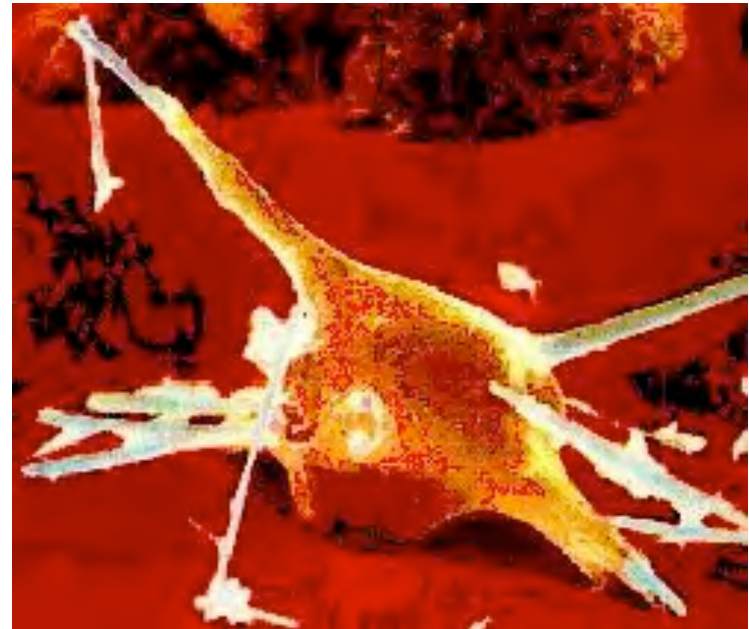


Fiber Toxicology



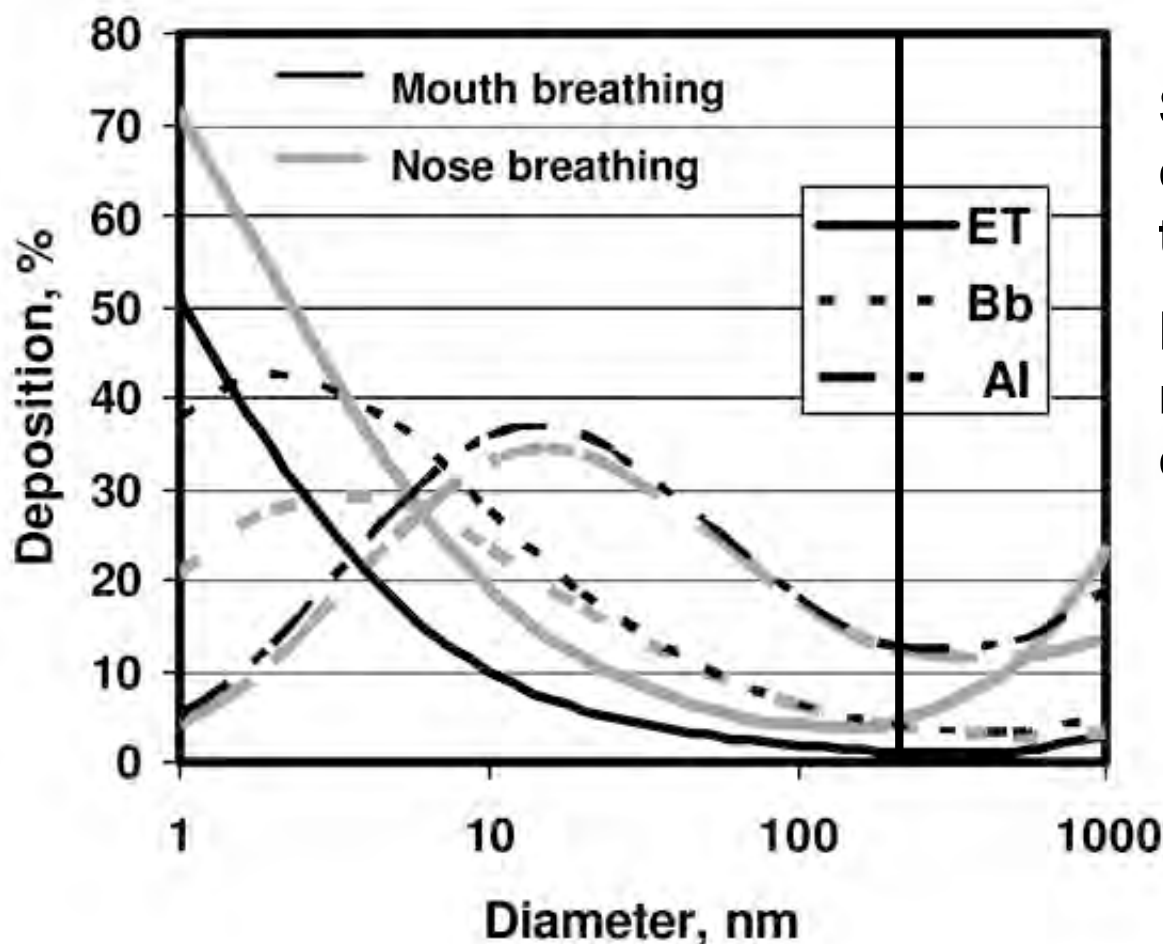
•Key factors contributing to toxicity

- Diameter < 1000 nm
- Length >5,000 nm:
- High biopersistence (low solubility)
- Poor pulmonary clearance



The three D's: Dose, dimensions and durability!

Respiratory Tract Deposition

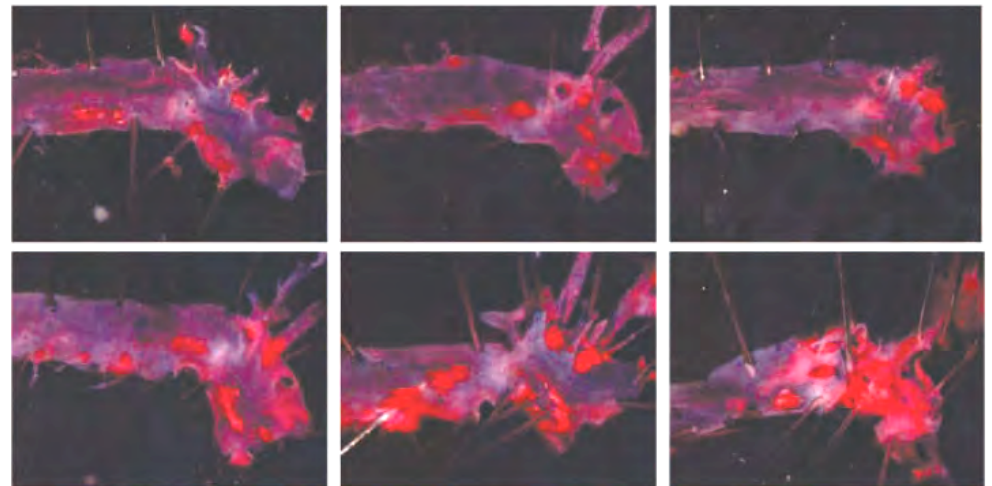
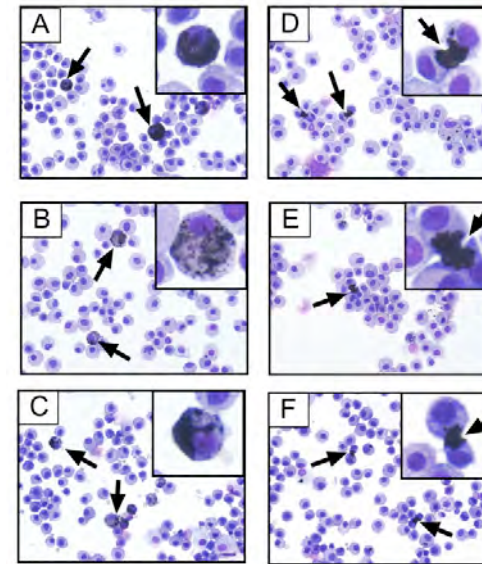
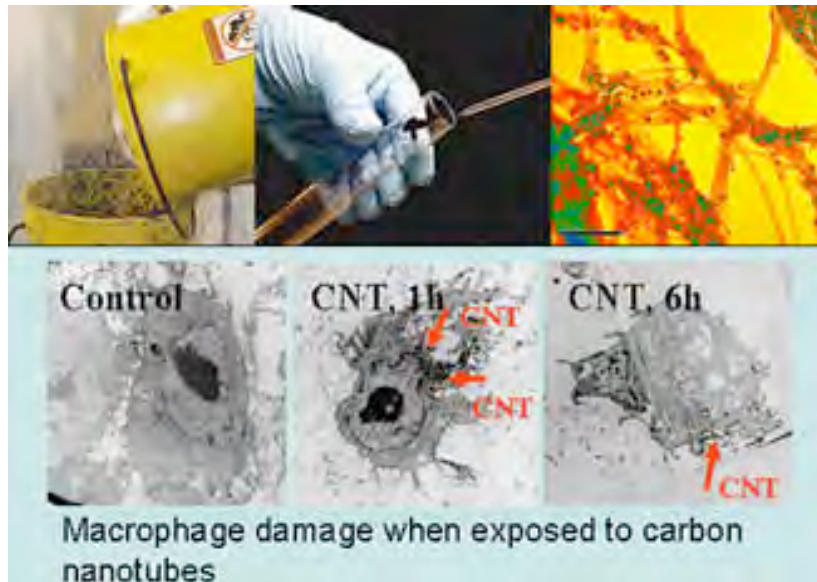


Shape and size
determine respiratory
tract deposition

Nanoparticle
respiratory tract
deposition curve

Borm et al. 2006,
based on ICRP 66

What has been published about carbon nanotube toxicity?



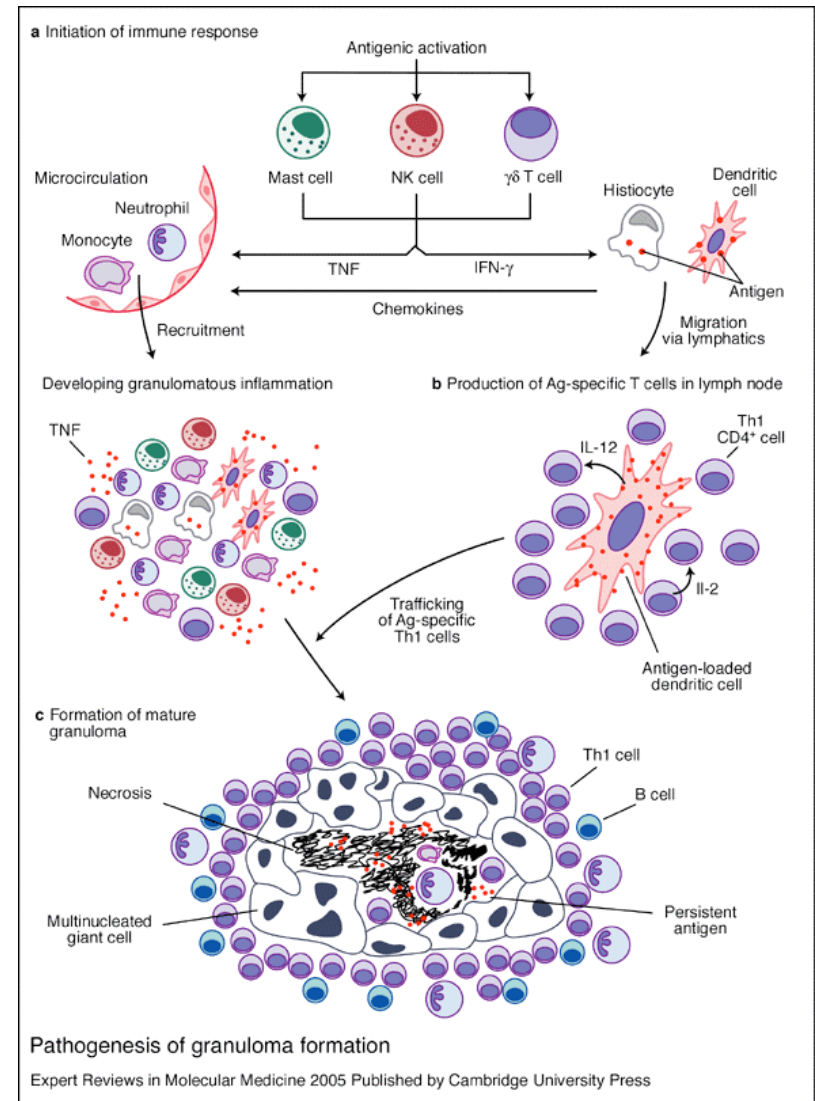
Parallel Literatures on toxicology of nanoparticles



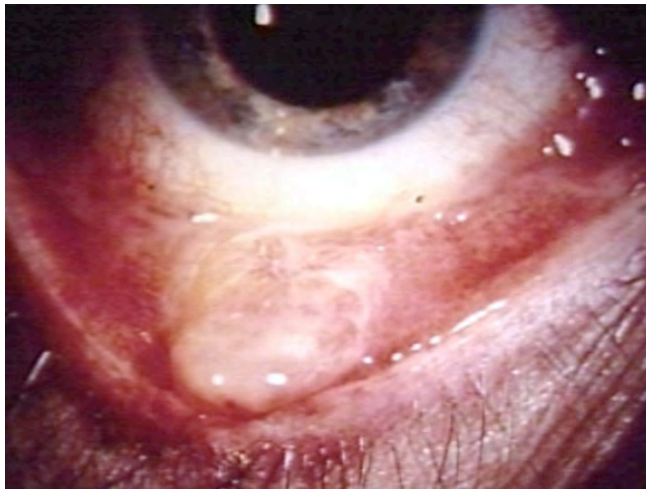
- Occupational/environmental toxicology
- Carbon nanotubes are being investigated for a host of medical uses, e.g. IV injection studies
- Air pollution scientists have been studying the health effects of “ultrafine” particles for many years

What is a granuloma?

- An inflammatory response that results when macrophages are unable to destroy foreign substances
- A tumor-like mass of inflammatory tissue consisting of a central collection of macrophages, often with multinucleated giant cells, surrounded by lymphocytes
- Seen in lungs in tuberculosis, sarcoidosis, berylliosis



External Granulomas



Occupational Toxicology Testing Methods



- **Inhalation>Instillation>In vitro**
- **Chronic>sub acute>acute**

The devil is in the details!



•Factors shown to influence CNT toxicity

- Concentration
- SWCNT vs. MWCNT
- Length
- Manufacturing method
- Catalyst residue
- Degree of aggregation
- Oxidation
- Functionalization
- Also species, dosing, assay, etc.

Selected cell culture studies



Year	Author	Cell Type	Conclusions
2003	Shevdova	Human skin fibroblasts	Oxidative stress and other signs of toxicity
2005	Ding (LBNL)	Human skin fibroblasts	MWCNT induce genes indicative of a strong immune, stress and inflammatory response
2005	Cui	Human embryonic kidney	SWCNTs inhibit cell growth by inducing cell apoptosis and decreasing cellular adhesion ability
2005	Jia	Human lung macrophages	SWCNT> MWCNT ₁₀ >quartz>> C ₆₀ Cytotoxicity
2005	Murr	Mouse lung macrophages	MWCNT “ropes” showed dose related cytotoxicity, more toxic than asbestos
2006	Tian	Human fibroblasts	Surface area predicts cytotoxicity, SWCNT>MWCNT
2007	Dvoren	Human lung	Very low toxicity in this assay
2007	Wick	Human mesothelioma	Nanoropes>asbestos>Dispersed CNT Cytotoxicity
2007	Pluscamp	Rat/human lung	Little acute cytotoxicity, ascribed effect to contaminants

Pulmonary Toxicity:

All published instillation/aspiration studies



Year	Lead Author	Type	Species	Conclusions
2001	Huczko	Vivo	Guinea pig	No effect on lung function detected
2004s	Warheit	Vivo	Rat	Some inflammation, non-uniform granulomas
2004s	Lam	Vivo	Rat	Granulomas, persistent inflammation
2005m	Muller	Vivo	Rat	Inflammation, fibrosis, granulomas CNT>asbestos>carbon black
2005s	Shevdova	Vivo	Mouse	Inflammation, oxidative Stress, progressive fibrosis, granulomas
2006s	Mangum	Vivo	Rats	Localized interstitial fibrotic lesions in alveolar region
2006m	Carrero-Sanchez	Vivo	Mice	Death with regular MWCNT via tracheal instillation, not with nitrogen doped MWCNT, did see granulomas. Less effects by other routes of exposure (nasal, oral)

That's all there is!

Problems with instillation/aspiration studies of CNTs



- **Many experimental problems:**
 - High dose in rodents choked the animals!
 - Particle aggregation may cause epiphenomenal localized toxicity (bronchial granulomas)
 - Some responses may be species specific
 - Contamination of CNTs with metals such as Mo, Ni used as catalysts during production may have effect
 - Nanotubes interfere with some biochemical assays of cell status

Huczko, 2001

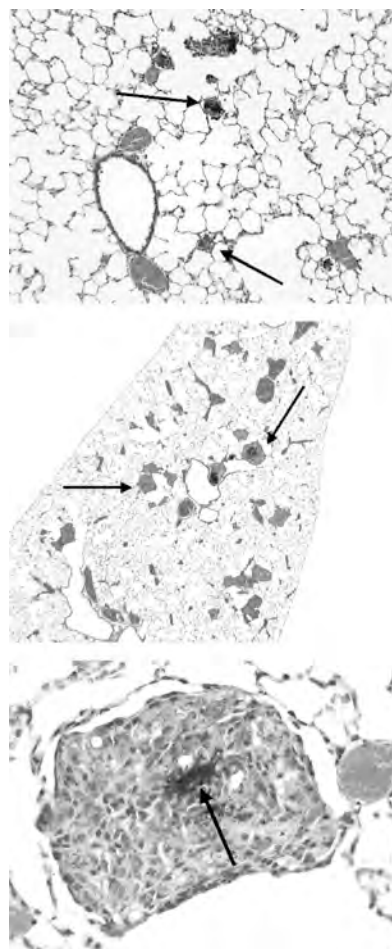


- Intratracheal instillation of soot with high or low levels of CNT into guinea pigs
- 25 mg, 4 weeks, # of animals not specified
- Measured breathing function and look at bronchial lavage fluids
- No effect seen, declared CNTs “*Unlikely to be associated with any health risk*”

Warheit, 2004



- Sparse granulomas
- Killed 15% of rats from suffocation
- Some inflammation
- Inconsistent results
- Believed results were epiphenomenal and not physiologically relevant



•SWCNT

•Intratracheal installation

•1 or 5 mg/kg body weight

•Rats

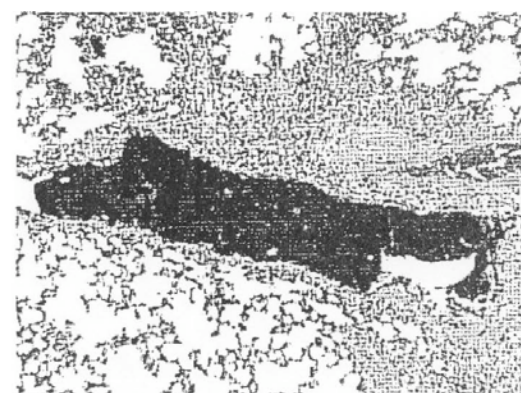
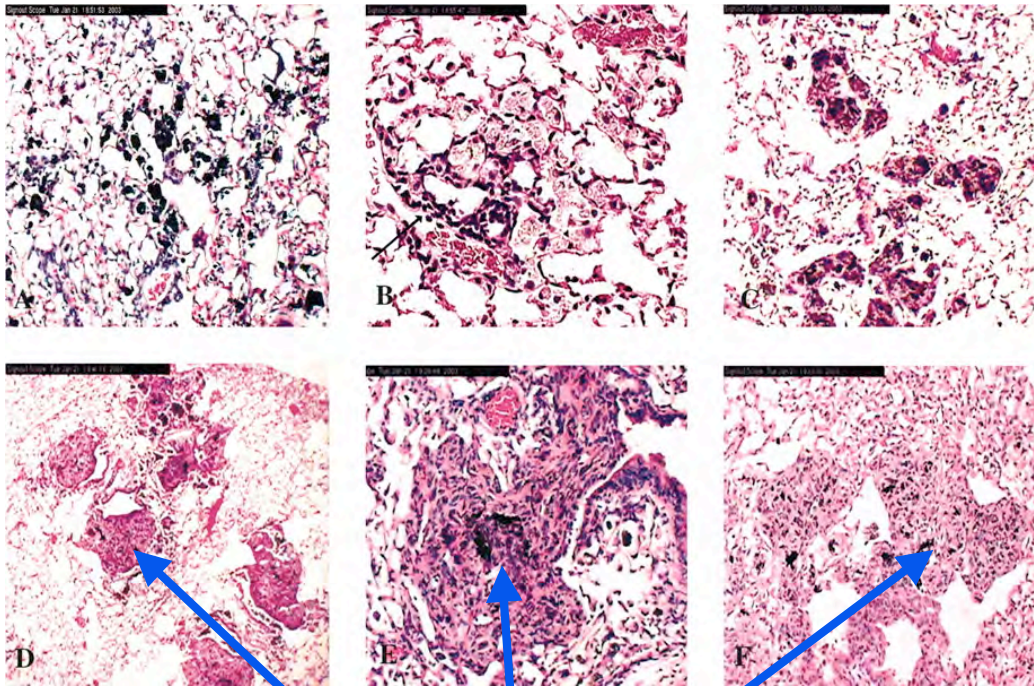


FIG. 1. Light micrograph of lung tissue from a rat exposed to 5 mg/kg SWCNT (a few h after exposure). The major airways are mechanically blocked by the SWCNT instillate. This led to suffocation in 15% of the CNT-exposed rats and was not evidence of pulmonary toxicity of SWCNT.

•Findings: persistent granulomas & inflammation

- A) Control
- B) Carbon black
- C) Quartz
- D) Raw electric arc CNT (Ni, Y catalyst)
- E) Raw HiPCO CVD CNT (Fe catalyst)
- F) Purified HiPCO CVD CNT (Fe catalyst)

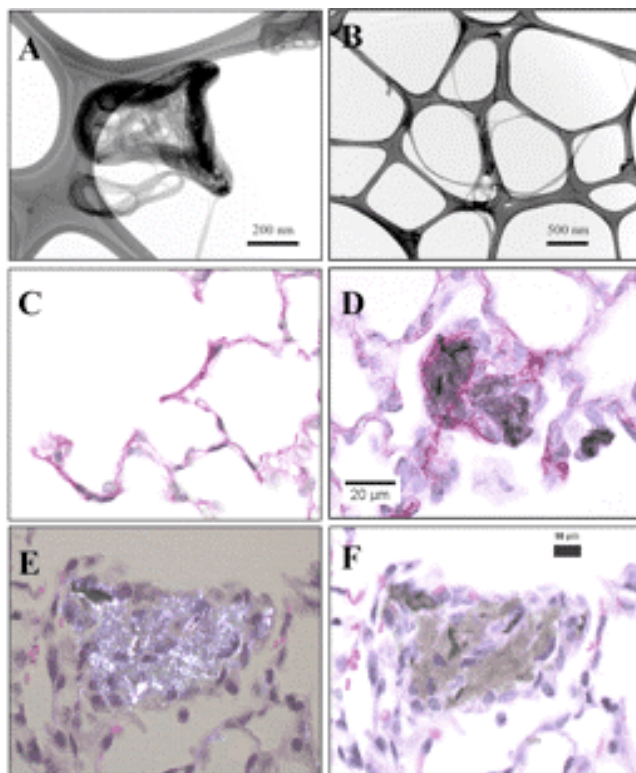


•CNT Granulomas

- 3 or 16 mg/kg body weight
- 7-90 days

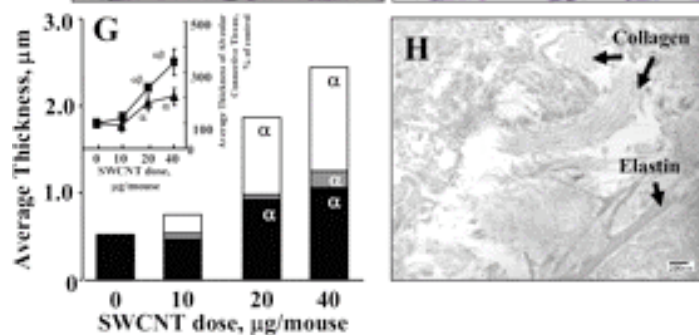
•Electric arc formed (Ni/Y catalyst) CNT killed many animals, HiPCO CVD (Fe catalyst) CNTs did not, but both caused similar granulomas

Shevdova, 2005



• Findings: inflammation, oxidative stress, granulomas where clumps landed, progressive fibrosis throughout lung

- A, B) Aggregated SWCNT by TEM on filter
- C) Lung section, control
- D) Lung section, exposed
- E, F) CNT in granuloma
- G) Dose-response fibrosis
- H) TEM image of fibrosis



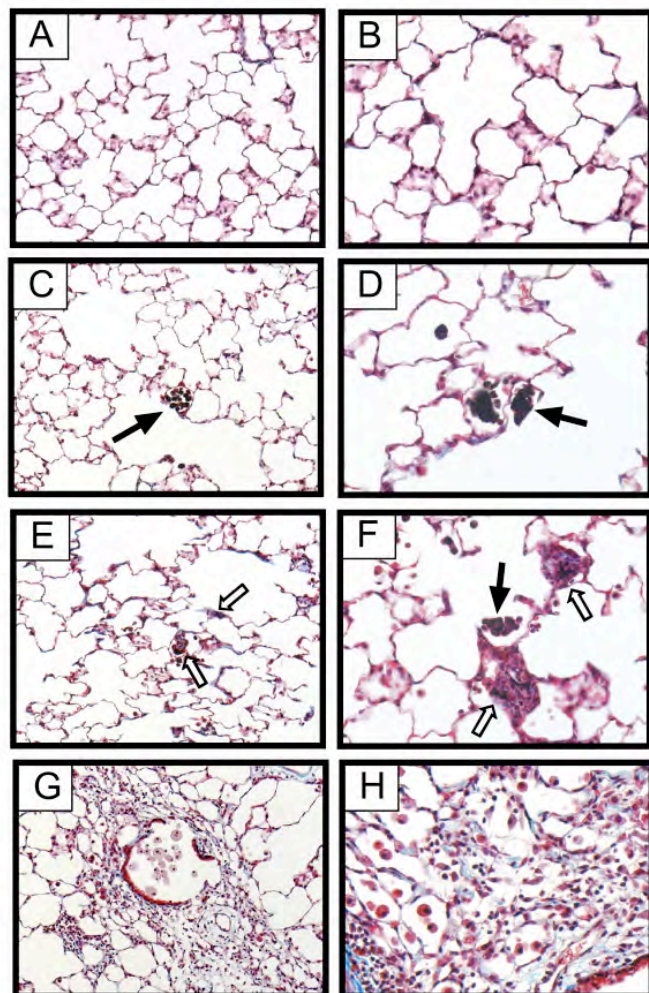
- Pharyngeal aspiration
- 0.5–2 mg/kg body weight
- Mice
- Highly purified SWCNTs
- 7-60 days

Muller, 2005



- **Intratracheal instillation of ground or unground MWCNT in rats**
- **Both forms caused inflammation and fibrosis**
- **2, 9, or 22 mg/kg body weight**
- **Ground MWCNT caused alveolar fibrosis, unground caused airway fibrosis**
- **More fibrogenic than chrysotile asbestos**
- **Studied out to 60 days**

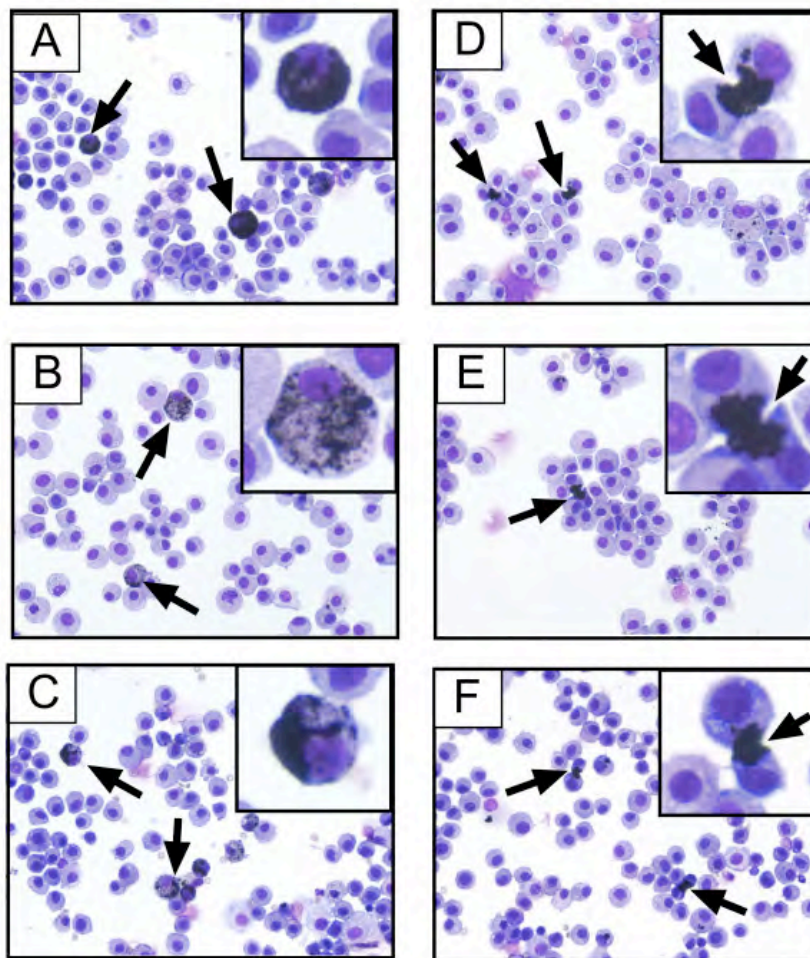
Magnum, 2006



- Saline vehicle (A, B)
- Carbon black ultrafine particles (C, D), aggregates of single walled carbon nanotubes, SWCNT (E, F), or vanadium pentoxide (V_2O_5) (G, H).
- **Regions of alveolar wall thickening in the lungs of rats exposed to SWCNT are indicated by open arrows adjacent to carbon-filled macrophages in panels E & F.**

- **No inflammation, only these scattered interstitial fibrotic lesions**
- **1-21 days**
- **Oropharyngeal aspiration**
- **2 mg/kg**

Magnum, 2006

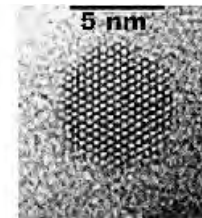


- “Bridges” between macrophages when exposed to CNTs (DEF), but not carbon black (ABC)

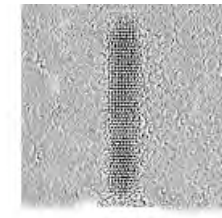
Distribution Across Anatomical Barriers



- **Nanoparticles may go where other particles can not!**
 - Through intact skin
 - Through the GI epithelium
 - Through the respiratory tract mucosal membrane
 - Through the bloodstream
 - Up along nerve axons from the nose to brain
 - Through the blood-brain barrier
 - Across the placenta



Spheres



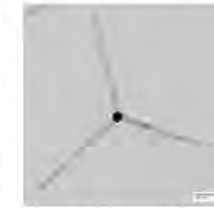
Rods



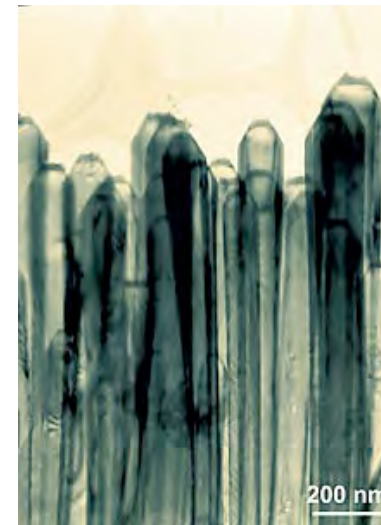
Wires



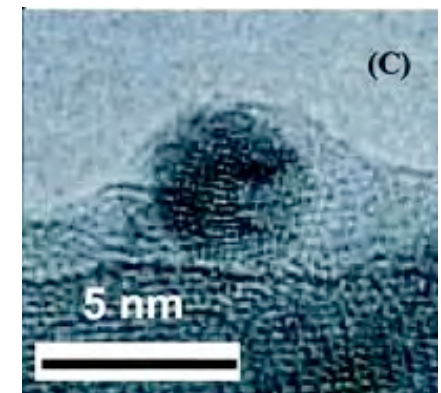
Ribbons



Tetrapods



Palladium



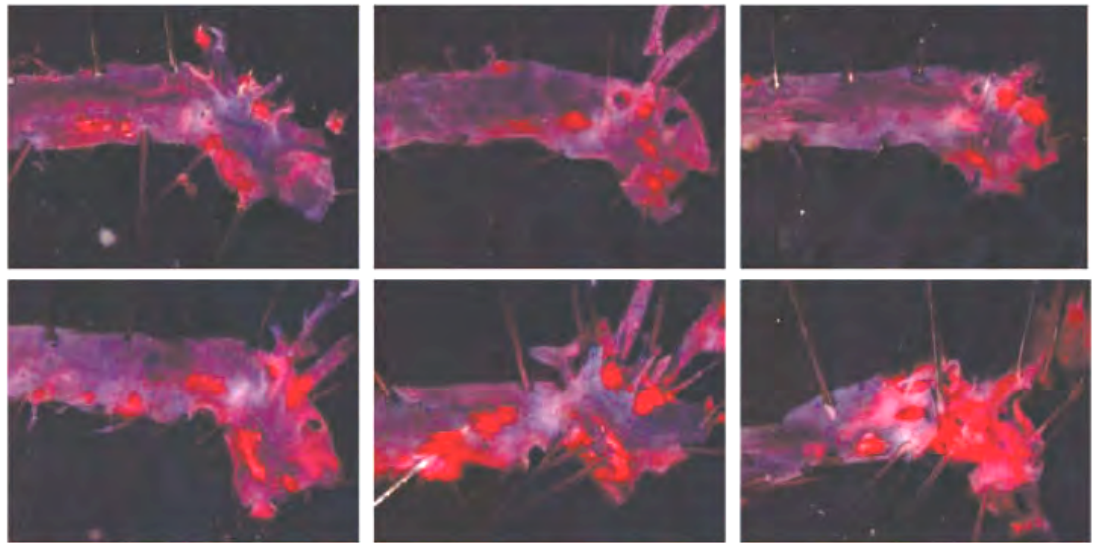
Cardiovascular Toxicity of CNTs instilled in the respiratory tract



- Ultrafine particles in polluted air have been associated with cardiovascular disease

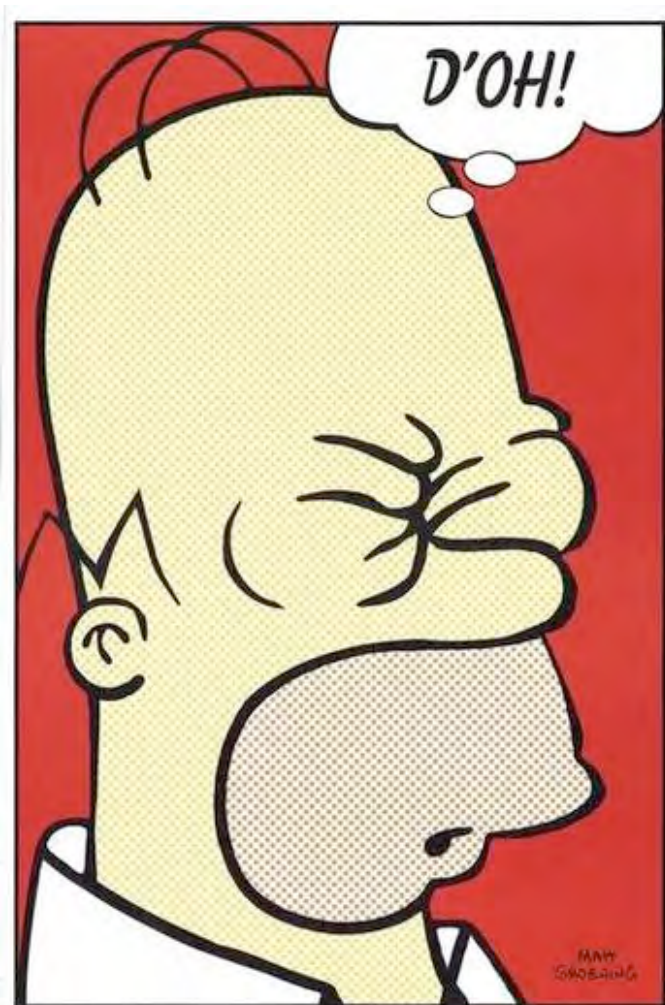
- Li, March 2007 (NIOSH)
 - intraparyngeal dosing of SWCNT in mice resulted in oxidative stress in lung, aorta and heart tissue and damaged mtDNA in aorta
 - Accelerated atherosclerosis

Control aortas



SWCNT exposed aortas

Inhalation Studies



- No good published CNT inhalation studies to date!

Cancer?



- **CNTs can enter cell nuclei**
- **DNA can interact with CNTs**
- **Some preliminary unpublished culture studies from NIOSH suggest that CNTs may intercalate with DNA, increase DNA bridging**
- **Nobody has studied this end point in animals**

But What is the Real Exposure Potential?



Raw single walled carbon nanotube material HiPCO Process)

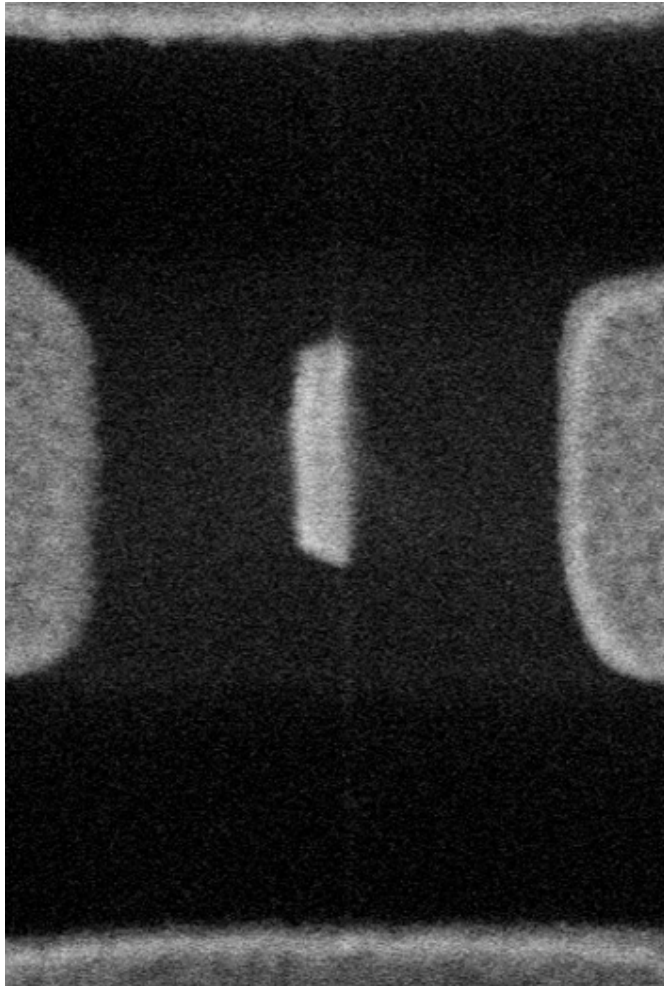
What does that mean?



Can't say much, but:

- Current Federal permissible exposure limit (PEL) for graphite dust is 5 mg/m³
- Shevdova and Lam estimated that disease may occur in humans exposed for ~1 month at PEL
- PEL for CNTs should be much lower than 5 mg/m³
- PEL should be expressed as particle concentration, not mass concentration (like asbestos)

Questions?



- **Nanotube-suspended motor, by Alex Zettl**